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青阳参种子的萌发

王定康[1,2]，孙桂芳[3]，翟书华[2]，岑晓江[2]，赵　剑[4]，萧凤回[1]

（1 云南农业大学中药材研究所 云南省中药材规范化种植技术指导中心，云南 昆明　650201；
2 昆明学院生物系，云南 昆明　650031；3 昆明医学院技能中心，云南 昆明　650031；
4 昆明市第十四中学，云南 昆明　650106）

摘要：青阳参（*Cynanchum otophyllum*）种子在 11 月成熟时有休眠习性。收获后将其种子种植在自然温室内，到第二年的春天种子才会萌发，且大多数种子在 3 月 28 至 4 月 4 日间萌发，这期间的日平均最高和最低温度分别为 19.0℃和 9.9℃。层积能有效地打破青阳参种子的休眠，休眠种子通过大约 1 周的层积便能萌发。种子在有光的条件下层积 1 周后转移到 25 15℃的黑暗条件下萌发率可达到 75.4%。青阳参种子不论在有光的条件下还是在黑暗环境中层积 2～3 周后转入 30 20 和 25 15℃进行变温处理，其萌发率最低能达到 66.4%，而转入 20 10℃变温处理其萌发率最多只能达到 20.1%，但若层积 6 周，即便在 20 10℃变温处理的情况下其萌发率也可以达到 65.3%以上。

关键词：青阳参；萌发；种子；层积；温度

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Seeds Germination of *Cynanchum otophyllum* (Asclepiadaceae) Endemic to China

WANG Ding-Kang[1,2]，SUN Gui-Fang[3]，ZHAI Shu-Hua[2]，CEN Xiao-Jiang[2]，
ZHAO Jian[4]，XIAO Feng-Hui[1**]

(1 *Institute of Chinese Medicinal Materials of Yunnan Agricultural University*，*Yunnan Provincial Center for Chinese Medicinal Material GAP Technology*，Kunming 650201，China；
2 *Biology Department*，*Kunming University*，Kunming 650031，China；
3 *Skill Center*，*Kunming Medical College*，Kunming 650032，China；
4 *Kunming No*. 14 *Middle School*，Kunming 650106，China)

Abstract：Intact seeds of *Cynanchum otophyllum* were innately dormant at maturity in November. Seeds planted in a non-heated greenhouse in November germinated the following spring and most of the germination occurred between 28 March - 4 April，when the mean maximum and minimum daily temperatures were 19.0 and 9.9℃，respectively. Stratification was effective in overcoming dormancy，and nondormant seeds germinated in both light and darkness. About one week of stratification was effective in overcoming dormancy in some of the seeds; seeds stratified in light for 1 week and then incubated in darkness at 25 15℃ germinated to 75.4%. Seeds stratified in light or darkness for 2 and 3 weeks and then incubated in light or darkness germinated to 66.4% or more at thermoperiods of 30 20 and 25 15℃ but germinated to 20.1% or less at 20 10℃. Seeds stratified for 6 weeks also germinated to high percentages (65.3% or more) at 20 10℃.

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Author for correspondence；E-mail：fenghuixiao@163.com

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作者简介：王定康 (1966-) 男，博士，教授，主要从事药用植物繁殖生物学研究。E-mail：wdk117@163.com

Key words: *Cynanchum otophyllum*; Germination; Seeds; Stratification; Temperature

Germination is a crucial phase in the life cycle of a plant, greatly affecting its fitness (Narbona *et al.*, 2007). In order for the seeds of a species to germinate, they must be in suitable environmental conditions, which usually favour the growth and establishment of the seedlings (Harper, 1977). However, viable seeds of some species do not germinate even though conditions are favourable. Five classes of dormancy are known to occur in seeds: morphological (MD), physiological (PD), morphophysiological (MPD), physical (PY), and combinational (PY + PD) (Baskin and Baskin, 2004).

Cynanchum is a large genus of some 400 described species with a tropical and subtropical distribution in Africa, Madagascar, Australia, North and South America, and parts of Asia (Liede, 1997). Seeds of most of the studied species of this genus have physiological dormancy (Sheeley, 1992; Zhou *et al.*, 2003; Wang *et al.*, 2007). Seeds of *C. rossicum* stored at 4.5℃ for 5 weeks in the dark had relatively high germination percentages under either light (46%) or dark (36%) conditions in a controlled environment chamber when subjected for 7 weeks to fluctuating temperatures of 13 - 21℃ (Sheeley, 1992). In contrast, seeds of *C. komaroviil* stored 2 - 4℃ for 40 d, and then incubated under an alternating temperature (25℃ 5℃; 12 h light dark), the percentage of germination was 3.33% (Zhou *et al.*, 2003).

Cynanchum otophyllum Schneid. is a native Chinese perennial vine found on open forest of upland and shrubs of hillside, ranging from Xizang to Sichuan, Guangxi, Hunan and Yunnan. The roots are used as medicine for rheumatism, epilepsy, hydrophobia, and detoxification of viper bites (Li *et al.*, 1995). Some information on the biology of *C. otophyllum* has been summarized (Li *et al.*, 1995). Little is known about the characteristics of *C. otophyllum* seeds.

One purpose of this study was to determine whether the freshly matured *C. otophyllum* seeds are dormant. The second purpose was to observe what the time seeds germinate when they are exposed to natural temperature regimes during autumn-winter and spring and what the temperatures are when germination occurs. The third purpose was to investigate the germination responses of seeds stratified in light and darkness for various periods of time and then incubated in light and darkness at four alternating temperature regimes which represent those occur in the field from early spring to summer.

1 Materials and methods

1.1 Seed collection site

The studied site of *Cynanchum otophyllum* was located on a hill with yellow soil near the town of Longchang, Xuanwei County, Yunnan Province, P. R. China (104°09′E, 26°11′N). The climate is subtropical with an annual average temperature of 14.1℃. The *C. otophyllum* plants were growing in a dry area, covering about 3 000 m^2 and usually thrive in patches. The surrounding landscape was a mixture of small woodlots, and agricultural crops. There were about 500 plants within the whole area.

1.2 General

We collected mature seeds from a population of *C. otophyllum* plants on 8 November 2006. The seeds were separated from the tufts of silky hairs. The collected seeds were allowed dry for 1 week in the laboratory, and the experiments were started on 15 November 2006. Seeds were considered to be 0 weeks old on that date. In all experiments radicle protruded 1 - 2 mm was the criterion germination.

1.3 Germination at natural temperatures

Each 250 seeds in two copies were planted on greenhouse potting soil in large flats and placed in a non-heated greenhouse in Kunming University′s Botany Experimental Field. The windows in this greenhouse were open during the entire experiment and temperatures were similar to those out-of-doors. Mean weekly maximum and minimum daily temperatures were calculated from continuous thermograph records made in the greenhouse. The soil in the flats was watered daily to keep it similar to field capacity. The flats were examined at about 1-week intervals, and each time the germinated seeds were counted and then removed.

1.4 Germination responses of stratified seeds

Seeds of *C. otophyllum* were placed on moist, quartz sand in Petri dishes (50 seeds in each of 240 dishes) and stratified at 4±2℃ for 1, 2, 3, 6 weeks. One half of the seeds was stratified in constant darkness, and the other half was stratified in light (12 h of 28.8 μmol m^{-2} s^{-1} of cool white fluorescent light each day). All Petri dishes were wrapped with wrap to retard loss of water, and with aluminum foil to provide constant darkness. At the end of the various stratification periods seeds were transferred to four alternating temperature regimes (15 6, 20 10, 25 15, and 30 20°C). These temperature regimes approximate the average daily thermoperiods that occur near the soil surface in spring and summer in Kunming. At each temperature, seeds stratified in light and darkness were incubated in light and darkness, and seeds stratified in darkness were incubated in light and darkness too. Non-stratified seeds (0 weeks of stratification) were placed in light and darkness at each temperature regime on 15 November 2006 at the beginning of the experiment. Seeds incubated in light received 12 h of 2 000 lux of cool white fluorescent light each day. The high and low temperatures were maintained for 12 h each day. Three replications (dishes) of seeds were used for each treatment. Germination percentages were determined for all seeds 2 weeks after they were placed at the alternating temperature regimes.

1.5 Statistical analysis

Mean germination percentage and standard error were statistically tested with Student's *t*-test (Zar, 1984).

2 Results

2.1 Germination at natural temperatures

Germination of the seeds sown after dried for 1 week was practically null. It began in 17 weeks about the date between 14 March and 21 March 2006 (Fig. 1), and during this time the mean maximum and minimum daily temperatures were 18.7 and 10.5°C (Fig. 1), respectively. Most of the seeds germinated between 28 March and 4 April. During this interval the mean weekly maximum daily temperatures ranged from 18.6 to 19.5°C, and the mean weekly minimum daily temperatures ranged from 9.8 to 10.0°C. The mean maximum and minimum daily temperatures from 21 March and 4 April were 19.0 and 9.9°C, respectively. The mean maximum of seeds germination percentage was 66.8±2.21% at natural temperatures.

2.2 Germination responses of stratified seeds

Freshly matured, non-stratified seeds (0 weeks) germinated to low percentages in both light (5.2±1.2%, 8.2±1.58%) and darkness (15.3±3.42%, 8.1±2.12%) at 25 15 and 30 20°C, but there was no germination at 20 10 and 15 6°C (Table 1). After 1 week of stratification, germination at 25 15 and 30 20°C was greatly improved, especially for seeds incubated in darkness, but there was no germination at 20 10 and 15 6°C. Regardless of whether seeds were in light or darkness during stratification or during incubation at the alternating temperatures, seeds receiving 2 weeks of stratification germinated to 66.4±3.78% or more at 25 15 and 30 20°C, and to 9.2±2.45% or less at 20 10; no seeds germinated at 15 6°C. Seeds that received longer stratification periods (3 and 6 weeks) showed an

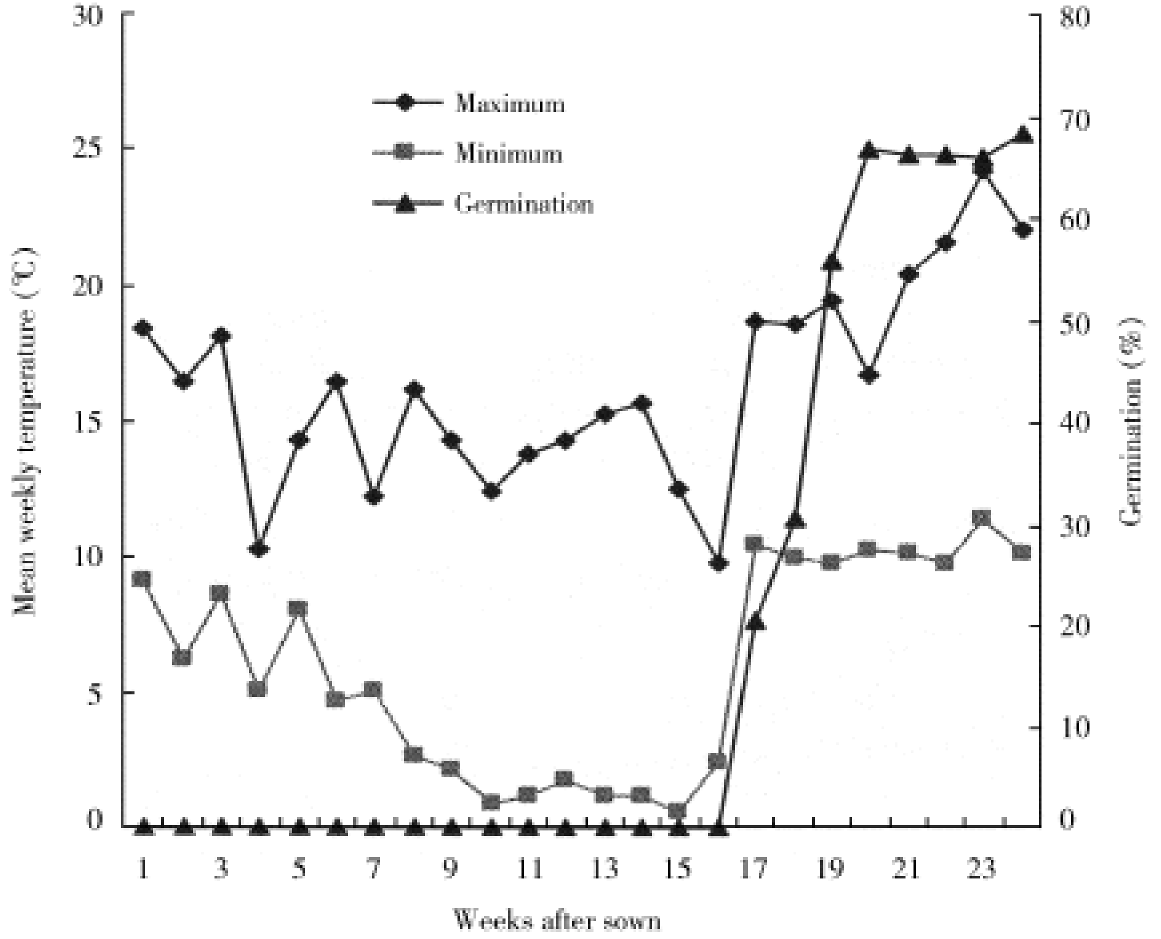

Fig. 1 Germination of *C. otophyllum* seeds planted on soil in a non-heated greenhouse in Kunming, Yunnan

Table 1 Germination percentages of *C. otophyllum* seeds after 0, 1, 2, 3 and 6 weeks of stratification. Seeds stratified in light were incubated in light and darkness, and seeds stratified in darkness were incubated in light and darkness (mean±s.d)

Stratification treatment	Germination temperature change (℃)	Stratification time (wks)				
		0	1	2	3	6
Light-Light	15 6	0	0	0	0	0
	20 10	0	2.80±1.21	4.1±0.78	20.1±1.32	76.5±1.32
	25 15	5.2±1.2	34.2±3.65	85.2±6.54	90.3±5.78	94.2±3.55
	30 20	8.2±1.58	20.0±1.65	75.3±4.64	89.2±3.96	94.1±1.34
Dark-Light	15 6	0	0	0	0	0
	20 10	0	0	4.8±1.21	7.5±1.87	78.5±0.77
	25 15	0	19.1±2.54	66.5±3.33	91.0±6.21	95.2±2.56
	30 20	0	21.4±2.45	68.7±5.23	85.1±5.54	95.3±2.23
Dark-Dark	15 6	0	0	0	0	0
	20 10	0	0	4.0±0.65	18.3±1.22	65.3±3.12
	25 15	15.3±3.42	65.2±5.75	75.5±6.58	90.1±4.66	95.2±3.28
	30 20	8.1±2.12	30.3±3.37	66.4±3.78	84.5±5.64	96.1±2.79
Light-Dark	15 6	0	0	0	0	0
	20 10	0	0	9.2±2.45	16.2±1.36	75.2±2.12
	25 15	0	75.4±3.45	90.1±6.23	92.5±5.21	97.1±1.63
	30 20	0	66.0±3.69	80.4±4.21	91.5±4.98	86.2±5.31

increase in ability to germinate at 20 10, 25 15, and 30 20℃. After 6 weeks of stratification, regardless of whether seeds were in light or darkness during and or after stratification, seeds germinated to 94.1±1.34% or more at 30 20 and 25 15℃ but no seeds germinated at 15 6℃. Seeds germinated to 55.3±3.12% or more incubated in darkness at 20 10℃.

3 Discussion

Seeds of *Cynanchum otophyllum* dried for 1 week at natural temperature did not germinate. However, the fact that the seeds sown under the same conditions did germinate after 17 weeks indicates that they had physiological dormancy. Physiological dormancy is common. It has been reported in common milkweed (*Asclepias syriaca* L.) (Evetts and Burnside, 1972; Jeffery and Robison, 1971; Oegma and Fletcher, 1972) and butterfly milkweed (*Asclepias tuberose* L.) (Bir, 1986) in the family of Asclepiadaceae. Dormancy can be broken by short period of moist, low-temperature treatment (stratification) (Evetts and Burnside, 1972; Jeffery and Robison, 1971; Oegma and Fletcher, 1972; Yang and Liu, 2005), removing the seed coat (Oegma and Fletcher, 1972; Ren and Abbott, 1992), dry laboratory storage (Evetts and Burnside, 1972) and treatment with gibberellic acid or kinetin (Evetts and Burnside, 1972; Oegma and Fletcher, 1972). Physiological dormancy also has been found in species of the genus *Cynanchum*, in which stratified at 2 - 5℃ for 5 - 7 weeks was effective in overcoming dormancy (Sheeley, 1992; Zhou *et al.*, 2003; Wang *et al.*, 2007).

When seeds of *C. otophyllum* are dispersed in November, although a small percentage of the seeds is non-dormant, there is no germination. These non-dormant seeds have a high temperature requirement for germination (30 20 and 25 15℃) (Table 1) and can not germinate in natural conditions because temperatures are too low then in Kunming. When the seeds begin to after-ripen during winter, they obtain the ability to germinate to high percentages at temperatures representative of late April and early May (25 15 and 30 20℃), but germination is prevented because temperatures of the environment are too low in the experiment site. With further after-ripening the seeds become capable of germinating if subjected to lower temperatures (20 10℃), but temperatures of the environment are still below those required for germination. Germination is delayed until spring when temperatures of the environment increase and correspond to those required for germination. Seeds subjected to natural, low winter temperatures in the non-heated greenhouse germinated in April when mean maximum and minimum daily temperature were about 20 and 10℃, respectively (Fig. 1).

In the early stages of after-ripening (after 1 week of stratification) seeds germinated better when incubated in darkness than in light (Table 1) . As the seeds became more fully after-ripened, however, they germinated equally well in light or darkness, regardless of whether or not they received light during stratification . Thus, seeds that overwinter in the field can germinate in darkness in spring if they become buried in either autumn or spring . Jeffery and Robison (1971) found that seeds germinated better when planted 1 or 2 cm deep than when planted on the soil surface . One possible reason for low seed germination in their study was the fact that they used open pots, causing high surface evaporation and low water availability to seeds .

To sum up, *Cynanchum otophyllum* seeds have non-deep physiological dormancy, which is broken in late spring and early summer, thus allowing seeds to germinate in the optimal wet season . The physiological dormancy of seeds can be broken by stratifying at a low temperature . Seeds can germinate under the situation of light and darkness .

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